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A Different Outlook on the Role of Bone Marrow Stem Cells in Vascular Growth

Bone Marrow Delivers Software not Hardware

Matthias Heil, Tibor Ziegelhoeffer, Barend Mees, Wolfgang Schaper

Blood vessel growth in adult organisms is a process occurring under various physiological and pathological conditions. Two forms of blood vessel growth have been described after birth: angiogenesis, ie, capillary sprouting, and arteriogenesis, ie, growth of large conductance arteries from preexisting arterial anastomoses (collaterals).[1,2] It is increasingly recognized that blood flow deficits caused by occlusion or stenosis of a major artery can only be efficiently compensated by arteriogenesis.

Fluid shear stress (FSS) is suggested to be the molding force for arteriogenesis. The activation of the collateral endothelium caused by increased FSS is reflected by an upregulation of adhesion molecules and release of cytokines that attract circulating blood cells, mainly monocytes, to adhere to and invade the collateral vessel wall.[3] Besides this monocyte/macrophage accumulation around growing collaterals, also an increase in numbers of T cells and granulocytes has been reported, underlining the paradigm that circulating cells are of a great importance in this type of vascular growth.[4,5] A variety of different cytokines and proteases (ie, MCP-1, FGF-2, TGF-β, uPA, and MMPs) produced by these invading cells have been identified to stimulate endothelial and smooth muscle cell proliferation and migration as well as tissue degradation.[6] This has led to a number of animal studies and clinical phase I trials demonstrating the short-term safety of administering several cytokines. However, recent clinical phase II trials have failed to extrapolate the promising results from animal studies into the therapeutic realm in human patients.[7,8]

New therapeutic approaches to promote arteriogenesis have evolved when it was suggested that the infusion of circulating bone marrow–derived stem or endothelial progenitor cells may improve blood flow recovery in various ischemic models.[9,10] The described positive effects on blood flow recovery were interpreted as a result of the incorporation of bone marrow–derived cells (BMCs) into growing capillaries. Even new definitions like neovascularization and neovasculogenesis were introduced. Since there is much evidence to suggest that the growth of true "bypass" arteries, arteriogenesis, is the most effective process to restore bulk blood flow to the affected tissue, the following crucial question in explaining the positive effects of circulating BMCs on blood flow recovery remains: "Can these effects be attributed to the incorporation of stem cells into the wall of new vessels or to the cytokines released by chemoattracted bone marrow cells inducing proliferation of resident endothelial and smooth muscle cells?" So far, this question remains a hot topic in vascular biology.

In this issue of *Circulation Research*, Kinnaird and collaborators[11] report interesting results suggesting that cultured human bone marrow–derived stromal cells (MSCs) promote arteriogenesis through paracrine mechanisms. The authors demonstrate that in MSCs in hypoxic conditions the expression of a panel of genes encoding for cytokines related to arteriogenesis is upregulated. In the MSC-cultured medium (MSC^{CM}), the presence of these cytokines (eg, MCP-1, VEGF-A, FGF-2, IL-6, PlGF, etc) was confirmed. When the medium was tested in vitro, endothelial cell and smooth muscle cell proliferation were increased. Moreover, in a murine hindlimb ischemia model, intramuscular injection of the MSC^{CM} improved collateral blood flow recovery and limb function and reduced muscle atrophy. Therefore, they conclude that not cell incorporation but paracrine signaling may be an important mediator of bone marrow cell therapy in tissue ischemia.

The positive arteriogenic effects of the MSC^{CM} in this study[11] can easily be explained by the components in the medium. Individually, all these cytokines, present in the MSC^{CM}, have been shown to have positive effects on blood flow recovery in experimental arteriogenesis.[12,13] Certainly, the approach to use cultured medium of BMCs as a "natural arteriogenic cocktail" is elegant, avoiding practical and ethical issues regarding cell therapy. Aside from these issues, the natural origin and composition of the solution as well as the combination of different cytokines could have an advantage above previous (single) cytokine treatments.

The authors seem to suggest that a trial with the injection of bone marrow–derived cells might also be warranted. Surely, it would have been interesting to compare the arteriogenic effects of the MSC^{CM} with the arteriogenic effects of the MSCs themselves, especially in the light of the key question mentioned above.

Nonetheless, the results of Kinnaird et al[11] definitely support findings from our laboratory. Recently, we have

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published a study strongly suggesting that in the adult organism BMCs do not promote vascular growth by incorporating into vessel walls but rather by acting as "cytokine factories."[4] In a hindlimb ischemia model in mice with reconstituted GFP-positive bone marrow, we failed to colocalize GFP signals with endothelial or smooth muscle cell markers. However, we observed strong accumulations of GFP-positive cells around growing collateral arteries, robustly expressing several growth factors and chemokines. These accumulated cells were mainly identified as leukocytes, which is in line with a previous study suggesting that endothelial progenitor cells originate from monocyte/macrophage lineage and secrete angiogenic growth factors.[14] Although these three studies[4,11,14] have different approaches, the same conclusion can be drawn, ie, stem cells promote vascular growth by their paracrine effects and not by incorporating into the wall of growing vessels.

These reports are in conflict with other studies evaluating the role of endothelial progenitor or bone marrow–derived cells in vascular growth.[9,10] In arteriogenesis, and also in atherosclerosis research, conflicting data on the incorporation of BMCs exist. In atherosclerotic lesions, incorporation of BMCs has been reported[15] and a reduction of plaque formation after BMC transplantation, suggesting renewal of the vessel wall.[16] In contrast, in another study an increase in atherosclerotic plaque size was observed after BMC transplantation under ischemic conditions, with only occasional incorporation of the transplanted cells into the plaque.[17]

Why do we get these different results? In studies reporting incorporation of BMCs into growing vasculature, these findings were based on the use of conventional light or fluorescence microscopy with substantial variations between magnifications. These microscopy techniques may not be sufficient to rule out "pseudo double-labeling," ie, immunostaining from two cells in close proximity to each other appearing to originate from a single cell. However, with high-power laser-scanning confocal microscopy, it is possible to differentiate a colocalization from an overlapping of signals due to convolution of different perivascular cells around cells of the vascular wall. In our study,[4] using this advanced method, we have not been able to find any evidence of integration of BMCs into the endothelium or tunica media of growing vessels. These conflicting findings prove to us that there is a need for more stringently controlled confocal studies aiming to localize multiple markers of cell lineage in order to evaluate the role of endothelial progenitor or bone marrow–derived cells in vascular growth. This need is even stronger with regard to all different approaches and cell types used in BMC transplantation, and the observation that the isolation and subsequent culture of progenitor/stem cells under special conditions could change the properties of these cells.

In conclusion, the study by Kinnaird et al[11] supports the idea that not the incorporation of BMCs into vessel walls is required for vascular growth in the adult but rather the paracrine effects of these cells. Stem cell therapy might be a promising tool in therapeutic vascular growth, however, not by building up an artery, but rather by supplying the logistics required for efficient arteriogenesis.

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KEY WORDS: arteriogenesis ■ bone marrow ■ vascular growth ■ cytokines